interface and the site corresponding to the AChE active site. This suggests that, while these mutations are unlikely to directly interfere with binding of NLs to Nrxs, they likely cause defects in NL folding, affecting its dimerization, thus indirectly altering binding to Nrxs. Alternatively, protein processing events may be altered in some cases (Dean and Dresbach, 2006).

Thus, the crystal structures reported here reveal key features that modulate *trans*-synaptic interactions between NLs and Nrxs, and therefore help elucidate how association of these complexes influences synaptic maturation and strength. These new findings provide insights into synapse specificity regulated by coupling of specific Nrx and NL variants and further our understanding of how mutations linked to ASDs may alter normal protein function.

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A Doubleheader in Endocytosis

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Synaptojanin1 degrades the signaling lipid phosphatidylinositol-4,5-bisphosphate and facilitates compensatory endocytosis, clathrin-coat disassembly, and vesicle reavailability at active synapses. In this issue of *Neuron*, Mani et al. provide new information about the separate roles of synaptojanin's two phosphatase domains and its interactions with endophilin in regulating these important aspects of the vesicle cycle.

Phosphoinositides such as phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] perform a wide array of tasks at the neuronal plasma membrane. At synapses, these regulatory lipids—which are rapidly metabolized by lipases, kinases, and phosphatases—have been implicated in both the exocytotic and endocytotic limbs of the synaptic vesicle cycle (for review, see Di Paolo and De Camilli, 2006). While much progress has been made in characterizing the functions of phosphoinositides in the presynaptic terminal, the precise actions of their metabolizing enzymes in the various aspects of the vesicle cycle remain unclear. In this issue of *Neuron*, Mani et al. (2007) examine the multiple roles played in synaptic vesicle cycling by a key element in phosphoinositide turnover, synaptojanin1 (Synj1), which is a polyphosphoinositide phosphatase that converts PtdIns(4,5)P_2 to PtdIns(4)P and PtdIns(4)P to PtdIns. Genetic ablation of *synj1* in mice (Cremona et al., 1999), worms (Harris et al., 2000), and flies (Verstreken et al., 2003) causes depressed synaptic transmission after prolonged stimulation, decreased synaptic vesicle numbers, and accumulation of clathrin-coated vesicles, suggesting that Synj1 regulates uncoating and remobilization of vesicles during clathrin-mediated endocytosis. In addition,

Harris et al. (2000) reported an increase in endocytic pits in *C. elegans* mutants, which raises the possibility that Synj1 may also have a direct effect on endocytosis. Mani et al. (2007) have now used optical reporters of synaptic vesicle trafficking in Synj1-deficient cortical neurons to show that Synj1 does indeed facilitate both compensatory endocytosis and vesicle reavailability, in addition to its well-characterized action on the uncoating phase of the vesicle cycle. This is similar to the conclusions of Dickman et al. (2005), who reported a strongly reduced rate of endocytosis at *Drosophila* neuromuscular junctions in the absence of synaptojanin. Furthermore, Mani et al. (2007) also characterized the separate roles of Synj1's distinct functional domains by analyzing the restoration of function produced by expression of modified Synj1 proteins in a *synj1*$^{-/-}$ background. They concluded that both of Synj1's phosphatase domains, as well as its proline-rich region where endophilin binds, are necessary for endocytosis, depending on the level of synaptic activity. Vesicle reavailability is also controlled by both phosphatase domains, although the interaction with endophilin plays a partial role as well.

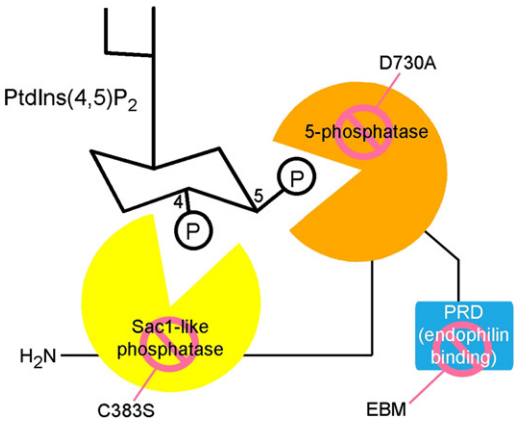

Figure 1. Synaptojanin1 Domain Structure and Loss-of-Function Mutations

Synaptojanin1 dephosphorylates phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] via a central 5-phosphatase domain (orange). The amino-terminal Sac1-like phosphatase domain (yellow) hydrolyzes PtdIns(4)P [as well as PtdIns(3)P and PtdIns(3,5)P_2] to PtdIns. Synaptojanin's proline-rich domain (PRD; blue) at the carboxyl terminus binds to endophilin and other proteins involved in endocytosis. The indicated single-residue substitutions (D730A and C383S) abolish activity of each phosphatase domain, and EBM (endophilin-binding mutant) indicates a constellation of five amino-acid substitutions that block interaction with endophilin.

Since mice lacking Synj1 typically die ~24 hr after birth, Mani and coworkers examined the roles of synaptojanin1 in the vesicle cycle by culturing cortical neurons from 1-day-old knockout mice. To track exocytosis and compensatory endocytosis, they used FM dye turnover (Betz and Bewick, 1992) or synaptopHluorin (spH), which consists of pH-sensitive GFP (pHluorin) fused to the lumenal end of the synaptic vesicle protein VAMP-2/synaptobrevin (Miesenböck et al., 1998). The acidic interior of resting vesicles quenches spH fluorescence, but when exposed to the neutral extracellular space upon exocytosis, spH fluoresces brightly. During compensatory endocytosis, fluorescence diminishes again as vesicles reacidify (Miesenböck et al., 1998). To separate exocytosis from endocytosis, reacidification was blocked with bafilomycin A1 (Atluri and Ryan, 2006), and the difference between fluorescence changes with and without bafilomycin was taken to represent the amount of endocytosis triggered by stimulation.

Cortical neurons lacking Synj1 exhibited less endocytosis than wild-type neurons during 30 s of stimulation at 10 Hz, as indicated by both spH fluorescence and FM dye uptake. Similarly, endocytosis following briefer stimulation (50–100 action potentials at 10 Hz) was slower and less complete in *synj1*$^{-/-}$ neurons. Expressing wild-type Synj1 in *synj1*$^{-/-}$ neurons reversed these effects. Since levels of PtdIns(4,5)P_2 can also influence *exo*cytosis, changes in endocytosis could potentially be secondary to increased exocytotic load in the absence of Synj1. However, Mani and colleagues concluded that this is not the case, because *synj1*$^{-/-}$ and wild-type neurons were indistinguishable in the rate of exocytosis (measured by changes in spH fluorescence in the presence of bafilomycin) and in the size of the cycling pool of vesicles (indicated by FM dye loading).

Building on the ability of wild-type Synj1 to rescue the endocytosis defect in *synj1*$^{-/-}$ neurons, Mani et al. next turned to the roles played by three different functional domains within the Synj1 protein, shown schematically in Figure 1. Synj1 is unusual in having two distinct phosphatase domains (the "two faces" that led to its name, after the god Janus): a Sac1-like inositol phosphatase domain near the amino terminus, and a central inositol 5-phosphatase domain. A third important region is the C-terminal proline-rich domain (PRD) that interacts with endocytic proteins, including endophilin. The amino-terminal Sac1-like phosphatase hydrolyzes PtdIns(3,5)P_2, PtdIns(4)P, or PtdIns(3)P to PtdIns (Guo et al., 1999), whereas the central 5-phosphatase hydrolyzes PtdIns(4,5)P_2 to PtdIns(4)P (Erneux et al., 1998). A recent study (Perera et al., 2006) revealed that the 5-phosphatase activity of the alternatively spliced Synj1 isoform, synaptojanin-170, is important for clathrin coat maturation in non-neuronal cells, but the importance of the phosphatase and proline-rich domains in synaptic vesicle endocytosis is not yet clear. Therefore, Mani et al. (2007) selectively mutated each of the three domains and expressed the mutant proteins in *synj1*$^{-/-}$ neurons. An inactive 5-phosphatase domain (D730A mutation; Figure 1) did not rescue the endocytic defect after either 50–100 action potentials or 300 action potentials, suggesting that 5-phosphatase activity facilitates endocytosis for both weaker and stronger stimuli. Sac1-like phosphatase activity was also necessary, but only for weaker stimuli (50–100 action potentials). Mutant Synj1 with decreased binding to endophilin (Figure 1) still rescued the endocytic defect for weak stimuli, but not strong stimuli (300 action potentials). Taken together, these results suggest that both of the phosphatase

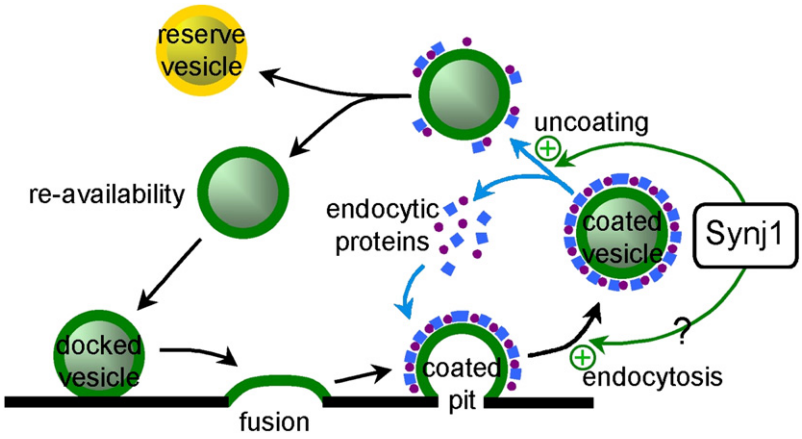

Figure 2. Model for the Involvement of Synaptojanin1 in Clathrin-Mediated Endocytosis

After a docked vesicle fuses with the plasma membrane, it is retrieved as a coated pit that eventually separates from the plasma membrane as a coated vesicle. The newly formed vesicle eventually sheds its clathrin coat and other endocytic proteins, which are then recycled in further rounds of endocytosis (blue arrows). The uncoated vesicle either enters a reserve pool or reenters the pool of vesicles available for exocytosis. Synaptojanin1 (Synj1) facilitates vesicle uncoating and may have additional, direct effects in promoting endocytosis (green arrows).

domains are necessary for efficient endocytosis, depending on stimulus conditions. Furthermore, interactions between Synj1 and endophilin facilitate membrane retrieval at high endocytic load, which underscores the importance of endophilin as a partner of Synj1 in regulating the synaptic vesicle cycle.

The physiological function of compensatory endocytosis is to resupply synaptic vesicles for subsequent neurotransmitter release. To determine which Synj1 domains are important for postendocytic vesicle reavailability, Mani et al. (2007) examined rerelease of FM4-64 (Ryan and Smith, 1995) in *synj1*$^{-/-}$ neurons expressing the domain mutants shown in Figure 1. Consistent with previous studies (Cremona et al., 1999; Harris et al., 2000; Kim et al., 2002; Verstreken et al., 2003; Dickman et al., 2005), rapid reentry of newly endocytosed vesicles into the releasable pool was impaired in Synj1-deficient neurons. Expression of wild-type Synj1 rescued the defect, while mutant proteins lacking 5-phosphatase or Sac1-like phosphatase activity did not, suggesting that both phosphatase domains are necessary for vesicle reavailability. Synj1 mutants unable to bind endophilin also partially rescued the defect. Because the binding mutation is specific for endophilin and the proline-rich domain also mediates additional protein-protein interactions, this partial effectiveness may indicate that other Synj1 partners are able to substitute in part for endophilin in facilitating vesicle reavailability.

Although it seems clear that Synj1 affects multiple parts of the synaptic vesicle cycle in mice, as it does in *C. elegans* (Harris et al., 2000) and *Drosophila* (Dickman et al., 2005), the mechanism by which Synj1 affects endocytosis and vesicle reavailability remains open. One possibility is that failure of uncoating is the primary defect in Synj1 null neurons, with impairments of endocytosis and vesicle reavailability following as secondary consequences. Figure 2 summarizes how facilitation of uncoating by Synj1 could lead to overall enhancement of endocytic throughput. In this scenario, the reduced rate of endocytosis in *synj1*$^{-/-}$ neurons stems from reduced availability of endocytic proteins (Figure 2, blue arrows) that get locked up in accumulated coated vesicles in the absence of normal PtdIns(4,5)P_2 turnover. Similarly, retarded uncoating might also give rise to slowed vesicle reavailability. In our view, this simple mechanism seems likely to explain at least part of the actions of Synj1 on endocytic rate, but it is not clear that it can account quantitatively for all of the details of the results. For instance, if impaired endocytosis arises from reduced availability of endocytic proteins, a straightforward prediction is that impairment should increase with endocytic load, but this was not always the case (e.g., absence of Sac1-like phosphatase activity was more detrimental for weak than strong stimuli). Therefore, it is reasonable to conclude that PtdIns(4,5)P_2 turnover by Synj1 may have additional, direct effects on the rate of endocytosis (Figure 2), such as promoting membrane curvature, membrane fission, or recruitment of other proteins to sites of membrane budding. Actions of Synj1 in processes other than clathrin disassembly are also suggested by studies of retinal cone ribbon synapses, where ribbons fail to anchor properly at active zones in *synj1* mutants (Van Epps et al., 2004).

Regardless of how many separate actions Synj1 may have, it is clear from the work of Mani et al. that the two phosphatase "faces" of synaptojanin 1 are crucial regulators of polyphosphoinositide turnover in the synaptic vesicle cycle. Given the broad importance of phosphoinositides in a variety of cellular processes involving protein targeting and vesicle trafficking, it will be interesting to see what similar roles other phosphatases—or possibly synaptojanin itself—may have in other functional contexts.

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Pumping Up the Synapse

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Synchronized control of excitatory and inhibitory synapse maturation is crucial for normal brain wiring, while its dysfunction leads to neurodevelopmental disorders, including autism. A paper in this issue of *Neuron* identified a novel role for the KCC2 pump, also responsible for the GABAergic synapse developmental switch, in regulating spiny excitatory synapse maturation, implicating it in the coordinated maturation of inhibitory and excitatory synapses.

Understanding the mechanisms of synapse development is a central issue in neurobiology, because synaptogenesis and developmental synaptic structural plasticity are crucial for the formation and refinement of neuronal circuits, including those underlying cognitive functions such as learning and memory, and because abnormal synaptogenic processes lead to neurodevelopmental disorders. Most excitatory synapses on pyramidal neurons in the mammalian forebrain are located on tiny mushroom-like protrusions called dendritic spines. During postnatal brain development, spine numbers and shape change dramatically, contributing to the formation and elimination of synapses, which in turn underlies the wiring and rewiring of neuronal circuits (Lendvai et al., 2000). During this period, spines also exhibit a high degree of structural and functional plasticity, which is associated with their maturation (Engert and Bonhoeffer, 1999). In young neurons, early in postnatal development, dendrites do not have spines, but are rather covered with thin, long, highly dynamic protrusions that often do not bear synapses, called filopodia. It is thought that filopodia participate in synapse formation by helping dendrites reach out for axons, thereby sampling potential presynaptic contacts (Lendvai et al., 2000). In more mature neurons, the morphology of existing spines is regulated by synaptic activity (Xie et al., 2007), and conversely, spine structure modulates synapse function. Activity-dependent spine plasticity therefore contributes to the experience-dependent refinement of neuronal circuits and to learning and memory. While filopodial and spinal plasticity is widespread during development, its extent in mature neurons is a subject of heated debate.

On the other hand, what is very clear is that numerous neurodevelopmental disorders that affect human cognition are associated with altered spine development (Fiala et al., 2002). Specifically, altered dendritic spine morphogenesis and maturation occurs in mental retardation, fragile X, autism spectrum disorders, Rett syndrome, Down's syndrome, Angelman's syndrome, neurofibromatosis, and tuberous sclerosis, just to name a few. This indicates that aberrant spine formation and remodeling are important pathophysiological factors in these disorders. Most importantly, in some forms of human nonsyndromic mental retardation, spine dysgenesis is the only detectable anatomical phenotype, testifying to the central role of spine morphogenesis in human cognitive development (Dierssen and Ramakers, 2006).

Dendritic spine morphogenesis, motility, and plasticity depend on regulated rearrangements of the synaptic actin cytoskeleton, as actin is very concentrated in spines and the actin cytoskeleton in spines is highly dynamic (Fischer et al., 1998). In addition, postsynaptic actin modulates glutamate receptor synaptic clustering and synaptic transmission and plasticity (Xie et al., 2007). Consequently, regulators of the actin cytoskeleton in spines are important for synapse maturation and plasticity.